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Abstract: Terrestrial ecosystems experience simultaneous shifts in multiple drivers of global change, which can interactively affect various resources. The concept that different resources co-limit plant productivity has been well studied. However, co-limitation of soil microbial communities by multiple resources has not been as thoroughly investigated. Specifically, it is not clearly understood how microbial communities respond to shifts in multiple interacting resources such as water, temperature, and nitrogen (N), in the context of global change. To test the effects of these various resources on soil microorganisms, we established a field experiment with temperature and N manipulation in three grasslands of northern China, where there is a decrease in precipitation from east to west across the region. We found that microbial responses to temperature depended upon seasonal water regimes in these temperate steppes. When there was sufficient water present, warming had positive effects on soil microorganisms, suggesting an interaction between water and increases in temperature enhanced local microbial communities. When drought or alternating wet–dry stress occurred, warming had detrimental effects on soil microbial communities. Our results also provide clear evidence for serial co-limitation of microorganisms by water and N at the functional group and community levels, where water is a primary limiting factor and N addition positively affects soil microorganisms only when water is sufficient. We predict that future microbial responses to changes in temperature and N availability could be seasonal or exist only in non-drought years, and will strongly rely on future precipitation regimes.

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Precipitation modifies effects of warming and N addition on soil microbial communities in northern Chinese grasslands

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Abstract Terrestrial ecosystems are experiencing simultaneous changes in multiple global change drivers, which can interactively affect multiple resources. The concept that multiple resources co-limit plant productivity has been well studied. However, co-limitation of soil microbial communities by multiple resources has not been as thoroughly investigated. Specifically, it is not clearly understood how microbial communities respond to multiple interacting resources such as water, temperature and nitrogen (N) in the context of global change. To test effects of these multiple resources on soil microorganisms, we established a field experiment with temperature and nitrogen manipulation in three grasslands of northern China, where precipitation shows a decreasing trend from east to west. We found that microbial responses to temperature depended upon seasonal water regimes in these temperate steppes. When water was sufficient, warming had positive effects on soil microorganisms, suggesting a strong interaction between water and warming affecting local microbial communities. When drought or alternating wet-dry stress occurred, warming showed detrimental effects on soil microbial communities.

Our results also showed clear evidence for serial co-limitation of microorganisms by water and N at the functional group and community level, where water is a primary limiting factor and N addition positively affects soil microorganisms only when water is sufficient. We predict that future microbial responses to temperature and nitrogen could be seasonal or exist only in non-drought years, and will strongly depend upon future precipitation regimes.

Key words: nitrogen, temperature, precipitation, co-limitation, soil microbial

community, temperate steppe

1. Introduction

Soil microorganisms require multiple essential resources (e.g., water, carbon (C), nitrogen, and phosphorus) for producing energy and synthesizing cellular macromolecules, and also depend on environmental factors such as temperature, soil moisture content, pH and salinity (Atlas and Bartha, 1998). It is crucial to better understand how these multiple resources and environmental constraints influence soil microbial communities in the context of global change. This is because global change drivers simultaneously and interactively alter multiple resources (IPCC 2007; Castro et al., 2010; Rousk et al., 2011) and potentially alter microbial mediation of ecosystem C and nutrient cycling. Thus, the influence of resources and environmental constraints on microbial communities may have far-reaching effects on ecosystem feedbacks to global change (de Vries et al., 2006 and 2007; Allison et al., 2010; Feng et al., 2010; Dijkstra et al., 2011; Zhang et al., 2011).

Optimal foraging theory predicts that organisms should allocate energy in such a way that they are equally limited by different resources in order to maximize net resource uptake per unit time (Bloom et al., 1985; Chapin et al., 1987). There have been various theories about how the simultaneous limitation of multiple resources or co-limitation occurs, including independent co-limitation at the level of the individual organisms (Saito et al., 2008) or populations or communities (Harpole et al., 2011). These theories serve as a framework from which to investigate the co-limitation by multiple resources, defined as successful utilization by organisms of one resource depending upon sufficient supply of another resource (Gleeson and Tilman, 1992;

78 Saito et al., 2008; Harpole et al., 2011). The definition of co-limitation has developed
79 from the single resource limitation of Liebig's law of the minimum (Liebig, 1855). At
80 the community level, Harpole et al. (2011) classified categories of co-limitation as
81 independent, simultaneous, or serial co-limitation. The community-level co-limitation
82 of microorganisms may simply reflect biochemical-level co-limitation only when all
83 species in a community are co-limited by the same resources. But community-level
84 co-limitation by multiple resources is usually quite complicated to detect or interpret,
85 due to species-specific physiological needs or adaptation (Schimel et al., 2007),
86 especially in the face of shifts in multiple nutrients and environmental constraints due
87 to global change.

88 Water occupies 70–90 % of the cell mass of microorganisms (Atlas and Bartha,
89 1998) and often co-limits soil microbial communities with other factors. A body of
90 evidence shows that warming and nitrogen (N) may interact with water fluctuation to
91 affect soil microbial communities, especially in arid and semi-arid ecosystems
92 (Allison and Treseder, 2008; Liu et al., 2009; Bi et al., 2011). Warming can reduce soil
93 microbial biomass and activities through water-stress on microorganisms (Zhang et al.,
94 2005; Rinnan et al., 2007; Liu et al., 2009; Rinnan et al., 2009), while improved water
95 availability may negate the negative warming effect, for instance through a
96 counter-intuitive increase in soil moisture under warming indirectly through plant
97 senescence (Zavaleta et al., 2003). This evidently shows an interaction between
98 warming and water affecting microbes. Grizzle et al. (2010) found that high water
99 availability can enhance the responses of soil microbial communities to N deposition.

100 This is not surprising because water physically influences or mediates microbially
101 mediated N processes (Schimel et al., 1996). For example, N addition can exert a
102 pronounced influence on soil microbial activities, but only under high water
103 availability (Bi et al., 2011), suggesting a serial co-limitation by water and N. In
104 addition, microbial communities from different climate regimes, and thus different
105 histories of adaptation, may respond differently to changes in either water or
106 temperature fluctuations (Schimel et al., 2007; Balser and Wixon 2009). We predicted
107 that the serial community-level co-limitation of water and N generally exists in
108 temperate grasslands given that water is a predominant limiting factor in temperate
109 ecosystems (LeBauer and Treseder, 2008; Liu et al., 2009) and is usually linked with
110 nitrogen availability or cycling rates (Schimel et al., 1996).
111 To test whether and how soil microbial communities are co-limited by multiple
112 resources, which largely and simultaneously shift in the context of global change, we
113 established a manipulative experiment with warming and N addition with continuous
114 treatment since April 2006 in three temperate grasslands of northern China. The three
115 temperate grasslands are along a decreasing natural precipitation gradient including
116 meadow (440 mm annual rainfall), semi-arid steppe (380 mm annual rainfall), and
117 desert steppes (313 mm annual rainfall). To further compare global change treatments
118 under natural weather regimes, we carried out measurements during two years with
119 contrasting levels of precipitation. This allowed us to examine how both local climate
120 regimes and natural fluctuations in weather from year to year alter microbial
121 responses to temperature and N addition. We hypothesized that these global change

drivers would alter multiple resources, among which (1) water and temperature could interactively influence soil microorganisms, (2) water and N could serially co-limit soil microorganisms at the community level, and (3) where differences could exist in microbial responses and in interactions between treatments between the three temperate steppes because of water deficiency in the semi-arid and the desert steppe and local adaption of microbial communities in the two drought ecosystems.

2. Materials and methods

2.1. Field sites

We conducted a field experiment in temperate grasslands of northern China. Concurrent changes in temperature, precipitation, and N deposition have been reported in the temperate zones of northern China (IPCC, 2007; Liu et al., 2007 and 2010; He et al., 2007; Zhang et al., 2008). Three grasslands including a meadow steppe, a semi-arid steppe, and a desert steppe, were included in this experiment. The meadow steppe is located in Changling County in southwestern part of Songnen Plain of Northeast China, and stands at the eastern edge of the Eurasian steppe (Fig.1 and Table 1). The semi-arid and desert steppes are situated in Duolun County and the Siziwang Banner of Inner Mongolia, respectively (Fig.1 and Table 1). All three steppes are in a continental temperate climate. There is a gradual decrease in mean annual precipitation from the meadow to the semi-arid, then the desert steppe (Fig.1 and Table 1). Soils are characterized as Chernozem with high sodic saline content in the meadow steppe, Haplic Calcisols in the semi-arid steppe and Kastanozem in the

144 desert steppe according to the Food and Agriculture Organization (FAO) classification
145 (Table 1). In comparison with the meadow and the desert steppe, the semi-arid steppe
146 has higher soil organic C and N, but lower soil pH (Table 1). The dominant plant
147 species in the meadow steppe are *Leymus chinensis*, *Puccinellia tenuiflora*,
148 *Calamagrostis epigeios*, *Chloris virgata* and *Suaeda glauca*. The vegetation of the
149 semi-arid steppe is dominated by *Stipa krylovii*, *Artemisia frigida*, *Potentilla acaulis*,
150 *Cleistogenes squarrosa*, *Allium bidentatum*, and *Agropyron cristatum*, while that of
151 the desert steppe is dominated by *Stipa breviflora*, *Artemisia frigida* and *Cleistogenes*
152 *mutica.*

153 *2.2. Experimental design*

154 The experiment was established in April 2006 and lasted for 4 years. Three sites,
155 i.e., a semi-arid, a meadow and a desert steppe, were used to set up plots with
156 manipulative warming or N addition. In the semi-arid steppe, we used a randomized
157 block design with 6 treatments including control (C), diurnal warming (W), N
158 addition (N), warming plus N addition (WN), daytime warming (6:00 a.m.- 6:00 p.m.)
159 and nighttime warming (6:00 p.m.- 6:00 a.m.) (Xia et al., 2009). All treatments were
160 replicated 6 times. Thirty-six 3×4 m plots were arranged in a 6×6 matrix. The distance
161 between two adjacent plots was 3 m. Among the six treatments, daytime and
162 nighttime warming are not used in this study in order to keep treatments in accordance
163 with the other two sites.

164 At the other two sites, we used a design with diurnal warming applied to blocks
165 and N addition applied to plots. We established twelve 3×4 m blocks, and kept

166 3m-distance between two adjacent blocks. Six blocks were warmed with an infrared
167 heater and the other six blocks were treated as unwarmed controls. N addition was
168 applied to 3×2 m plots within each block.

169 At all three experimental sites, one infrared heater (165cm × 15cm MSR-2420,
170 Kalglo Electronics Inc., Bethlehem, PA, USA) was suspended 2.25 m above the
171 ground in each warmed plot. The heaters were set at approximately1600 W radiation
172 output. In order to simulate the shading effects of the heater, 'dummy' heater
173 installations with the same shape and size were also set up in unwarmed plots.
174 Another continuous 4-year experiment provided strong evidence that plants saturated
175 at an N addition rate of 10.5 g N m^{-2} y^{-1} in temperate grasslands of Inner Mongolia
176 (Bai *et al*., 2010). Based on this evidence, N addition was applied once a year at a rate
177 of 10 g N m^{-2} y^{-1} using NH_4NO_3 in the early growing season of each experimental
178 year.

179 *2.3. Sampling and measurements*

180 Three soil cores (2 cm in diameter and 15 cm in depth) were collected from each
181 plot of the three sites at the peak of the growing season of August 2006 and 2007. Soil
182 cores were mixed into one composite fresh sample per plot to avoid bias from spatial
183 heterogeneity. After removing plant roots and stones with a 2-mm mesh sieve, soil
184 samples were immediately cooled with ice blocks and transported to the laboratory for
185 further analysis.

186 Soil organic carbon was estimated by dichromate oxidation and titration method
187 (Kalembasa and Jenkinson, 1973). Soil samples were digested using the Kjeldahl

188 acid-digestion method, and then Kjeldahl digests were analyzed for ammonium
189 concentration on an Alpkem autoanalyzer to determine total soil N (Kjektec Systerm
190 1026 Distilling Unit, Sweden). Soil was dried at 105 ºC for 48 h to measure water
191 moisture. Soil pH was measured using a glass electrode (1:2.5 soil to water ratio,
192 Thermo Orion T20, USA).
193 Soil microbial community composition was estimated using phospholipid fatty
194 acid (PLFA) analysis. This method avoids requirement for microbial culture, and can
195 assess most natural populations in soil microbial communities (Amann et al., 1995).
196 We extracted, fractionated and quantified PLFAs from fresh soils following the
197 procedure of Bossio and Scow (1998). Fresh soil sample equivalent to 8 g dry mass of
198 soil was extracted for 2 h using a single phase mixture containing chloroform,
199 methanol and phosphate buffer (1:2:0.8 v/v/v). After the supernatant was decanted,
200 soil was extracted again for 30 min. The twice-obtained supernatants along with
201 additional phosphate buffer and $CHCL_3$ were decanted into a separation funnel. After
202 phases were allowed to separate overnight, the organic phase ($CHCL_3$ layer) was
203 obtained and dried under N_2. After reconstitution, fatty acids were separated using a
204 silica-bonded phase column eluted first using 5 ml chloroform, 10 ml acetone and 5
205 ml methanol successively in order to separate polar lipids from neutral and glycolipids.
206 Polar lipids were converted to fatty acid methyl ester via mild alkaline methanolysis.
207 A 2 µl sample of each fatty acid methyl ester extract was injected and analyzed by an
208 Agilent 6850N gas chromatograph with a flame ionization detector and an HP-1 Utra
209 2 capillary column (Agilent Technologies, Inc., Santa Clara, CA, USA). Gas

chromatography was performed as recommended by the MIDI standard protocol (Microbial ID. Inc., Newark, DE). Peak areas of each resulting fatty acid methyl ester were recorded on a chromatogram and identified by chromatographic retention time and comparison with peaks from a standard qualitative mix ranging from C9 to C30 using a microbial identification system (Microbial ID. Inc., Newark, DE). The mole percentage (mol %) of PLFAs was expressed as the relative concentration of each PLFA in initial soil extracts. The PLFAs i15:0, a15:0, i16:0, a17:0, i17:0, 16:1ω7c, 18:1ω5c, cy17:0 and cy19:0 were used as the biomarkers of bacteria (Frostegård and Bååth, 1996; Zak et al., 1996; Ringelberg et al., 1997; Zelles, 1997; Zogg et al., 1997). Terminally branched saturated PLFAs i15:0, a15:0, i16:0, i17:0 and a17:0 were used as indicators of Gram-positive bacteria (Gram+), while cy17:0, cy19:0 and16:1ω7c were considered as indicators of Gram-negative bacteria (Gram-) (Frostegård and Bååth, 1996; Zak et al., 1996;Ringelberg et al., 1997; Zelles, 1997; Zogg et al., 1997). The unsaturated PLFAs containing 18:1ω9c, 18:2ω6c and 18:3ω6 were used as biomarkers of fungi (Zak et al., 1996; Ringelberg et al., 1997; Zelles, 1997; Zogg et al., 1997; Madan et al., 2002; Pinkart et al., 2002). 16:1ω5 was used to represent arbuscular mycorrhizal fungi (AMF; Olsson et al., 1995).

Three out of the six experimental replicates from the sites of meadow and semi-arid steppes were randomly chosen to perform the measurement of soil microbial C utilization. Soil microbial C utilization was measured using Biolog redox technology, which is a culture-based method for detecting soil microbial C source utilization, especially the C utilization of quick-growing, r-strategist species (Garland

232 and Mill, 1991; Gomez et al., 2006; Mijangos et al., 2006). The procedure was
233 performed according to Classen's description (Classen et al., 2003). After shaking for
234 30 min on a reciprocal shaker, 4 g of fresh soil were extracted with 36 ml of 50 mM
235 K_2HPO_4 buffer. The supernatant was obtained by settling for 30 min. The supernatant
236 was then diluted 1:1000 with a sterile incubation solution in order to prepare the
237 bacterial suspension. The sterile incubation solution consisted of 0.40% NaCl and
238 0.03% Pluronic F-68. 150 µl of bacterial suspension were injected into each well of
239 the EcoPlate. Each EcoPlate was put into a polyethylene bag to avoid desiccation and
240 incubated in darkness at 25°C. The optical density (OD) values were read at 595 nm
241 every 24 h. Given fungal detection after 96 h in a preliminary experiment, the OD
242 values at 96 h were used to assess bacterial C utilization for avoiding bias induced by
243 fungal growth. The net OD values were calculated as the substrate OD subtracted
244 from the control well OD. If the result was negative or less than 0.06, the net OD
245 would be considered as zero or below the detection limit of the system (Miguel et al.,
246 2007). The mean net OD of all substrates in each guild was calculated to assess
247 bacterial utilization of each C source guild. Average well-color development (AWCD)
248 was used to reflect bacterial metabolic potentials, and determined as follows (Garland
249 and Mills, 1991):

250 $$\mathrm{AWCD} = \sum_{i=1}^{n} (x_i - c)/31,$$

251 where x_i is the OD value in the substrate well, and c is the OD value measured in
252 the control well

253 *2.4. Data analysis*

Statistical software R 2.15.3 was used for conducting all statistical analyses (R Development Core Team, 2013). Given that the warming effect was applied to the blocks in the semi-arid steppe and to plots in the other two experimental sites and the otherwise fully balanced design without missing values, we used the 'aov' function with Error() function because this allowed us to conveniently separate the contribution of warming to variation in the dependent variables between block and plot level. Note that this type of design can not easily be handled by other mixed-model approaches such as the ones implemented in the R-functions ‘lme’ or ‘lmer’. Using the 'aov' function allowed us to test the warming effect and its interaction with year occurring in the semi-arid steppe at the block level, but in the other two sites at the plot level. The effect of Naddition was always tested at the plot level.

We analyzed the phospholipid fatty acid composition of soil microbial communities combining data from all experimental plots from all sites using principal component analysis (PCA). The PCA was conducted using the ‘rda’ function of the vegan package in R. To reduce noise for PCA, only 18 PLFAs with a mol % (moles individual lipid/moles total lipids) > 0.5 were used. We also performed post-hoc permutations using the function ‘envfit’ of the vegan package to detect associations of the microbial community composition determined by phospholipid fatty acid profiles with environmental variables. The function ‘cor.test’ of the stats package of R was used to perform Pearson's correlation test for associations between microbial functional groups and environmental variables.

We analyzed the matrix of net OD values from soil microbial C utilization

analysis also using PCA to test the responses of microbial C utilization profiles to warming and N addition using data from all experimental plots and all sites. We also performed permutation tests for associations between microbial C utilization profiles of r-strategist bacteria and environmental variables, similar to that performed for lipid data.

3. Results

3.1. Soil parameters

There were similar differences in soil organic C and total N in the plots between the meadow, semi-arid, and desert steppe sites in both sampling years unless otherwise noted (2006 and 2007; Table 2 and S1). Soil organic C and total N were greater in the semi-arid steppe than those in the meadow steppe and in the desert steppe (TableS1). The ratio of soil C to N was, on average, the highest in the meadow steppe (Table 2 and S1). Soil pH was the highest in the meadow steppe as well, followed by the desert steppe and then the semi-arid steppe (Table 2 and S1). Soil moisture between the three experimental sites was significantly different, but dependent upon year. Larger magnitude of site difference in soil moisture between the meadow and semi-arid steppe was observed in 2006 rather than in 2007 (Table S1).

The effect of N addition on soil organic C was significantly dependent upon site (Table 2). There was the greatest increase in soil organic C induced by N addition in the meadow steppe (Table 3). N addition pronouncedly reduced soil pH across the three temperate steppe sites, but the effects of N addition on soil pH were year- and

site-dependent (Table 2 and 3). We found that the negative effects of N addition on soil pH were the strongest in the meadow steppe in 2006 in comparison with the other two sites, but showed the strongest in the semi-arid steppe in 2007 (Table 3). Moreover, the negative effects of N addition on soil pH were stronger in 2007 than 2006 in both semi-arid and desert steppes (Table 3). Warming effects on soil pH were year-dependent as well (Table 2). There were large contrasting effects of warming on soil pH between 2006 and 2007 in the meadow steppe. However, we did not find main warming effects or interactive effects between warming and N addition on soil parameters in any of the three temperate steppe sites (Tables 2 and 3).

3.2. Microbial community composition as indicated by PLFA profiles

We displayed and used the first and second principal components (PC), which explained 40.2% and 25% of variance in phospholipid fatty acid composition, respectively. Although one principal component analysis (PCA) was performed, we display the PCs separately for each site in order to emphasize differences in microbial responses to treatment at each site. The PCA biplot also displayed clear separation of warming, N addition, and warming plus N addition from the control in 2006 (Fig. 2, top), indicating significant impacts of warming and N addition on microbial PLFA composition in this year in the meadow steppe. According to the loading scores which quantify the contribution of each individual fatty acid to the first two principal components, terminally branched saturated PLFAs i15:0, i16:0, a15:0 and saturated PLFA16:0, unsaturated PLFAs 16:1ω5c, 16:1ω7c, 18:1ω9c and 18:2ω6c largely contributed to changes in soil microbial community composition in the meadow

steppe. Warming and N addition had no effects on microbial PLFA composition in the semi-arid and the desert steppe, but there was still clustering by sampling year (Fig. 2, middle and bottom).

3.3. Microbial functional groups as indicated by specific PLFAs

Microbial functional groups included here, as determined by specific PLFAs, include total bacteria, Gram-negative (Gram-) and Gram-positive bacteria (Gram+), non-mycorrhizal fungi, and arbuscular mycorrhizal fungi (AMF). Arbuscular mycorrhizal fungi were pronouncedly different among the three sites with the largest proportion in the meadow steppe (Table 4 and S2). The largest proportion of non-mycorrhizal fungi was observed in the desert steppe site in 2006, but in the semi-arid steppe site in 2007 (Table 4 and S2). The trends of the fungal/bacterial ratio mirrored the trends of non-mycorrhizal fungi, because there was no difference of total bacteria among the three sites in both 2006 and 2007 (Table 4).

Although there were no main effects of warming or N addition on bacterial groups (i.e., total bacteria and Gram+), non-mycorrhizal fungi, or AMF across the three sites, we did observe interactions of year×N addition and year×site×N addition affecting the relative proportion of microbial groups (Table 4). This suggests that the effects of N addition were both year-dependent and ecosystem specific. The contrasting effects of N addition on microbial functional groups in 2006 and 2007 were especially noticeable in the meadow steppe site, for the total bacteria, Gram+, non-mycorrhizal fungi (Fig. 3, right uppermost panel). In the meadow steppe, N addition largely stimulated the proportion of total bacteria, Gram-, Gram+, Gram-/Gram+ ratio and

342 non-mycorrhial fungi in 2006 with a wetter growing season (Fig. S1, upper panel). In
343 contrast N addition greatly decreased total bacteria, Gram+, non-mycorrhizal fungi
344 and fungal/bacterial ratio in 2007 with a drier growing season (Fig.S1, upper panel).
345 N addition also showed the contrasting effects on bacteria, Gram+, fungi and AMF
346 between 2006 and 2007 in the desert steppe (Fig. 3, right lowest panel). Similar to N
347 effects in the meadow steppe, warming increased the proportion of total bacteria,
348 Gram-, Gram+, non-mycorrhizal fungi and AMF in 2006 and reduced them and the
349 fungal/bacterial ratio in 2007 (Fig. 3, left uppermost panel). Warming had negative
350 effects on microbial functional groups in the semi-arid steppe, and the warming
351 effects were stronger in 2006 than 2007 (Fig. 3, left middle panel).

352 *3.4. The microbial C utilization profiles and average metabolic potentials*

353 Principal component analysis (Fig. 4) showed that sample scores between 2006
354 and 2007 were arranged in two distinct groups in the meadow steppe site and were
355 marginally separated in the semi-arid steppe site. This implies that C utilization
356 profiles of r-strategist bacteria had large inter-annual fluctuation in both meadow and
357 semi-arid steppe sites. According to PCA analysis, we found bacterial C utilization
358 profiles in the plots of warming plus N addition clearly separated from the control
359 only in 2006 in the meadow steppe site, and those in warming and N addition plots
360 were also marginally different from the control (Fig. 4, upper panel). However, we did
361 not observe significant effects of warming, nitrogen, or any of their interactive effects
362 on bacterial C utilization profiles in the semi-arid steppe site (Fig. 4, lower panel).

363 The average metabolic potentials (AWCD) of r-strategist bacteria in the control

plots in the meadow steppe were larger than those in the semi-arid in 2006 (F = 123.6 (1), $P< 0.001$), but there was no significant difference between the two sites in 2007. Bacterial AWCD showed significant difference between 2006 and 2007 in both meadow and semi-arid steppe sites. Bacterial AWCD across treatments was 59.8 % (F = 141.02 (1), $P< 0.001$) higher in 2006 than that in 2007 in the meadow steppe site, but was 227 % (F = 30.08 (1), $P< 0.001$) lower in 2006 than in 2007 in the semi-arid steppe. Bacterial AWCD declined with N addition by 15.7% (F = 5.6 (1), $P =0.031$) in both years in the meadow steppe site, but was not altered by N addition in the semi-arid steppe site. There were no significant warming effects or interactions between warming and N addition on bacterial AWCD in either the meadow or semi-arid steppe.

3.5. Correlations between soil and microbial parameters

According to *post-hoc* permutation testing, soil microbial community composition closely correlated with soil organic C (r = 0.26, $P = 0.005$) and N (r = 0.24, $P = 0.019$), soil moisture (r = 0.65, $P< 0.001$), and pH (r = 0.56, $P< 0.001$). Across all plots and 2 experimental years, Gram+ was marginally correlated with soil organic C/N ratio (r = 0.14, $P = 0.099$). Gram- was marginally related to soil organic C (r = 0.16, $P = 0.059$) and N (r = 0.16, $P = 0.051$), while Gram-/Gram+ was marginally related to soil organic C (r = 0.15, $P = 0.065$) and was significantly correlated with soil total N (r = 0.26, $P = 0.002$). The mole percentage PLFA of total bacteria was only marginally correlated with soil moisture (r = 0.14, $P = 0.086$). The non-mycorrhizal fungal PLFA was positively correlated with soil organic C (r = 0.19, $P = 0.019$), but negatively

correlated with pH (r = - 0.15, P = 0.076). The fungal/bacterial ratio was significantly associated with organic C(r = 0.22, P = 0.009) and N (r = 0.19, P = 0.025), and marginally correlated with soil pH (r = -0.15, P = 0.082). Arbuscular mycorrhizal fungi were pronouncedly related to organic C (r = 0.24, P = 0.004), N (r = 0.17, P = 0.042), soil moisture (r = 0.59, P< 0.001) and soil pH (r = 0.40, P< 0.001).

According to permutation test, soil microbial C utilization profiles were closely correlated with soil organic C (r = 0.58, P = 0.002) and N (r = 0.50, P = 0.002), organic C/N ratio (r = 0.37, P = 0.039), soil moisture (r = 0.82, P< 0.001) and pH (r = 0.80, P< 0.001). Across all plots and 2 experimental years, the average metabolic potentials (AWCD) of r-strategist bacteria showed significant correlation with soil moisture (r = 0.78, P< 0.001) and pH (r = 0.48, P< 0.001), but not with soil organic C, N and C/N ratios.

4. Discussion

4.1. Annual precipitation-modified effects of warming on soil microbial communities

Liebig's law suggests that there is a single limiting factor to control the growth of organisms (Liebig, 1855) and the expanded concept of this theory is co-limitation, or the simultaneous limitation of more than one resource (Saito et al., 2008; Harpole et al., 2011; Ågren et al., 2012). In the present study, we found that annual precipitation showed great modification of warming effects on soil microbial communities, especially soil fungi in soils of the meadow steppe. Our results showed that in the meadow steppe, microbial functional groups were stimulated by warming or N

addition only under the conditions of high water availability in 2006, but not under the drought environment in 2007. In this co-limited system, water appears to be the primary limiting resource and the limitation of temperature as a second resource occurred only under high water availability, which implies a serial co-limitation of water and temperature at the functional group level of microbial communities (Harpole et al., 2011). The PCA results indicate that the serial co-limitation may also exist at the community level in the meadow steppe.

The primary limitation of water on soil microbial communities is easily understood. Water stress usually creates unfavorable growing conditions for soil microbial communities and makes the majority of microorganisms inactive, which may result in insensitivity of microbial communities to warming (Sheik et al., 2011). Alternatively, soil microbial phylotypes from historically more water-stressed environments may be better adapted to the drought environment and may also have strong inherent resistance to water stress (Schimel et al., 2007; Bradford et al., 2008). This may explain why soil microbial groups did not show significant responses to warming in either study year (2006 and 2007) in the semi-arid and the desert steppe sites. However, warming should most likely stimulate microbial growth when water is sufficient. High temperatures that do not kill microorganisms usually lead to higher enzyme activities and nutrient assimilation (Atlas and Bartha, 1998; Allison et al., 2010). Microbial populations surviving in sufficient water conditions are more active in comparison with those under drought conditions (Sheik et al., 2011), and different microbial populations may exhibit different temperature responses for different

430 temperature growth ranges (Atlas and Bartha, 1998). This may explain why the serial
431 co-limitation of microorganisms by water and temperature was observed not only at
432 the functional group level but also at the community level in our study.

433 *4.2. The effect of alternating wet-dry regimes or extreme drought on microbial*
434 *temperature responses*

435 Alternating wet-dry regimes or extreme drought create a large challenge and
436 physiological stress for microorganisms (Austin et al., 2004; Schimel et al., 2007;
437 Zeglin et al., 2013). We observed when an alternating wet-dry regime (during July and
438 August of both 2006 and 2007 in the semi-arid steppe) or extreme drought (during
439 August of 2007 in the meadow steppe) occurred around sampling time, most of the
440 microbial functional groups showed negative responses to warming. Warming could
441 reduce water availability, nutrient availability, or supply of root exudates, all factors
442 that work together to alter microbial population dynamics (Atlas and Bartha, 1998;
443 Austin et al., 2004) and also alter microbial community composition, because
444 different microorganisms have different inherent or acclimation abilities in response
445 to water stress (Zhang et al., 2005; Rinnan et al., 2007; Schimel et al., 2007; Evans
446 and Wallenstein, 2012). We found that warming exerted a stronger suppression on
447 non-mycorrhizal fungi relative to bacteria under the extreme drought condition in the
448 meadow steppe. This appears to be inconsistent with the general view that bacteria are
449 more largely dependent upon water and more sensitive to water stress while fungi
450 tend to be drought-tolerant (Wardle, 2002; Hawkes et al., 2011; Yuste et al., 2011). We
451 suggest that the high salinity and alkaline nature of soils in the meadow steppe may

452 complicate the responses of microbial communities to the drought stress that was
453 aggravated by warming because saline-alkaline soils are strongly detrimental to
454 fungal growth (Rousk et al., 2009; Djukic et al., 2010; Rousk et al., 2011). An
455 alternative explanation is that most of bacteria in saline-alkaline soils of the meadow
456 steppe were halotolerant or halophilic (Pan et al., 2012), and these bacteria could
457 better tolerate (e.g., excluding the high and toxic sodium ion concentration from their
458 cell interiors) or even require higher salt concentration (Atlas and Bartha, 1998), and
459 thus these bacteria relative to fungi in saline-alkaline soils of the meadow steppe may
460 have larger resistance to the drought regimes that warming aggravated.

461 *4.3. Precipitation-dependent N limitation of soil microbial communities*

462 Water and N are both limiting resources for plant productivity (Knapp et al., 2001;
463 LeBauer and Treseder, 2008) and could co-limit plant growth (Harpole et al., 2007).
464 In this study, we further found that most of the microbial functional groups positively
465 responded to N addition only without water stress. This implies that water is the
466 primary limiting factor relative to nitrogen for microorganisms, suggesting a serial
467 co-limitation of microbial communities by water and N in the temperate steppes of
468 northern China. Water is essential for nutrient diffusion and replenishment into the
469 soil solution. High water availability could contribute to the replenishment of added-N
470 into soil solution and consequently increase available N for microbial use (Park et al.,
471 2002). This could explain why N addition stimulated bacteria in our system (Wardle
472 et al., 2002). The accumulation of soil organic carbon under N addition appeared to
473 lead to an increase in fungal relative abundance in 2006 in the meadow steppe site,

consistent with the other observations (Cusack et al., 2011). However, a change in soil organic carbon due to N addition cannot explain the significant decrease in fungal relative abundance in 2007 in the meadow steppe. The drought in 2007 may have limited carbon and nutrient availability, weakening N effects on soil available nutrients and thus on fungi as well. Alternatively, drought could allow the accumulation of nitrate or ammonium ions (Stursova et al., 2006), even possibly to inhibitory levels for some extracellular enzymes, further inhibiting the growth and activity of fungi (Donnison et al., 2000). Taken together, our results indicate that N effects on soil microbial communities are strongly precipitation-dependent in the temperate steppe sites of northern China.

4.4. Water dependence of treatment effects on bacterial C utilization profiles and average metabolic potentials

Clear elucidation of microbial physiology is required for effective integration of microbial ecology with ecosystem ecology (Schimel et al., 2007; Balser and Wixon, 2009; Allison et al., 2010). Despite some limitations of the culture-based Biolog redox technology, Biolog has been proven a useful fingerprinting method of microbial C use physiology (Balser and Wixon 2009). We found that the bacterial C utilization profiles and the average metabolic potentials of r-strategist bacteria were largely correlated with soil moisture, consistent with the other observations (Bell et al., 2008).This can contribute to explain the finding that the great water fluctuations between sampling years caused the significant inter-annual differences in the bacterial C utilization profiles and the average C metabolic potentials in both meadow and

semi-arid steppes. Microbial growth and metabolic activities require water (Young and Ritz, 2005; Liu et al., 2009) and depend upon favorable water conditions to function. High soil water availability can provide for improved microbial growth and metabolic activities (Zak and Kling, 2006; Keeler et al., 2009; Liu et al., 2009), especially for bacteria, because they are particularly sensitive to soil water conditions (Degens et al., 2000; and as described above). The water dependence may also explain why much stronger treatment effects on the C utilization of bacterial communities were shown in the wetter 2006 in comparison with 2007 in the meadow steppe. In the semi-arid steppe, however, we found resistance of the bacterial C utilization profiles and their average metabolic potentials to treatments. Bacterial adaptation to historically drought conditions is a possible explanation.

5. Conclusions

Soil microbial communities in the semi-arid and the desert steppe appear to show more resistance to warming and N compared to those in the meadow steppe. The adaptation of microorganisms existing under drought environments in both semi-arid and desert steppes may a possible explanation for the site-different responses of microbial communities. Our results further showed that annual precipitation modified warming effects, or even co-limited soil microorganisms at the functional group and community level in the meadow steppe, not merely interactively affecting them as we proposed in our first hypothesis. We suggest that water is the primary limiting resource in these steppe ecosystems, and that temperature as the second limiting

518 resource has positive effects on soil microorganisms only when water is sufficient. In
519 addition, we found that alternating wet-dry regimes and extreme drought regimes
520 modified microbial temperature responses. In line with the second hypothesis, water
521 and N co-limited soil microorganisms. Following this, N addition increased microbial
522 functional groups and altered microbial community composition in the meadow
523 steppe only under high water availability and showed no or negative influences when
524 water was limiting. Overall, our results highlight high precipitation-dependent
525 temperature and nitrogen responses of soil microbial communities in the temperate
526 steppes of northern China.
527

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534

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Table 1 Geographic, climate and soil parameters in the meadow, the semi-arid and the desert steppe site. MAT= mean annual air temperature from 1959 to 2007, MAP = mean annual precipitation from 1959 to 2007. Seasonal precipitation in 2006 and 2007 refers to precipitation in the growing season from May to October each year. Means of soil parameters (mean ± standard error, n =12) in the control plots across 2006 and 2007 are shown in the table.

Sites	Meadow steppe	Semi-arid steppe	Desert steppe
Geographic coordinates	44°.45' N, 123°.45' E	42°.02' N, 116°.17' E	41°.47' N, 111°.53' E
Elevation (m a.s.l)	160	1324	1456
MAT (°C)	5.6	2.4	3.6
MAP (mm)	440	380	313
Seasonal precipitation in 2006 (mm)	348	404	193
Seasonal precipitation in 2007 (mm)	219	186	253
Soil type	Chernozem	Haplic Calcisols	Kastanozem
Soil organic C ($g \cdot kg^{-1}$)	9.13 ± 0.76	14.13 ± 0.78	10.96 ± 0.65
Soil total N ($g \cdot kg^{-1}$)	0.95 ± 0.09	1.45 ± 0.05	1.13 ± 0.07
Soil C/N ratio	10.04 ± 0.60	9.74 ± 0.51	9.27 ± 0.35
Soil pH	9.34 ± 0.18	7.21 ± 0.08	7.98 ± 0.04
Soil moisture (%)	14.41 ± 0.84	8.82 ± 1.14	5.60 ± 0.47

764 Table 2 Results (*F* values) of four-way ANOVA on the effects of site, warming, N
765 addition and year on soil physicochemical parameters. SOC = soil organic carbon, TN
766 = total soil nitrogen, C/N = the ratio of soil organic carbon to nitrogen, SM = soil
767 moisture, pH = soil pH. The degrees of freedom (d.f.) for the numerator, mean squares
768 (MS) and mean squared error (Residuals) are shown in the table as well. Significant
769 code ^, *, **, and *** refer to $P < 0.1$, < 0.05, < 0.01 and < 0.001.

Variance of source		SOC		TN		C/N		SM		pH	
	d.f.	MS	F	MS	F	MS	F	MS	F	MS	F
block											
site (S)	2	172.53	19.70***	0.69	7.82**	40.62	8.89**	954.60	137.35***	47.90	207.63***
warming (W)	1	0.12	0.01	0.04	0.41	11.39	2.49	8.00	1.15	0.08	0.33
S × W	2	7.70	0.88	0.05	0.59	0.02	0.00	0.45	0.06	0.02	0.09
Residuals	25	8.76		0.09		4.57		7.00		0.23	
block:plot											
W	1	1.31	0.18	0.05	0.57	1.23	0.20	1.12	0.23	0.00	0.00
N addition (N)	1	28.13	3.95^	0.20	2.07	11.08	1.81	8.09	1.66	1.79	9.26**
S × N	2	63.25	8.88**	0.25	2.58^	10.97	1.79	4.87	1.00	0.32	1.67
W × N	1	9.28	1.30	0.01	0.13	14.86	2.43	7.28	1.49	0.01	0.07
S × W × N	2	13.80	1.94	0.14	1.44	11.41	1.86	11.62	2.38	0.01	0.03
Residuals	35	7.12		0.10		6.13		4.89		0.19	
block:year											
year (Y)	1	83.48	32.95***	2.12	23.55***	28.31	7.17*	8.40	1.89	0.12	0.88
Y × S	2	15.93	6.29**	0.26	2.89^	12.24	3.10^	356.10	79.93***	1.26	9.36**
Y × W	1	0.07	0.03	0.05	0.53	0.00	0.00	1.60	0.35	0.64	4.74*
Y × S × W	2	2.16	0.85	0.06	0.70	4.95	1.25	0.45	0.10	0.41	3.07^
Residuals	25	2.53		0.09		3.95		4.50		0.13	
block:plot:year											
Y × W	1	0.08	0.02	0.07	0.78	11.78	1.44	0.08	0.03	0.00	0.01
Y × N	1	2.18	0.45	0.02	0.27	9.54	1.17	2.43	1.04	0.15	5.79*
Y × S × N	2	2.16	0.45	0.02	0.24	2.15	0.26	2.08	0.90	0.10	3.68*
Y × W × N	1	0.98	0.20	0.00	0.05	3.86	0.47	2.45	1.06	0.02	0.72
Y × S × W × N	2	1.42	0.30	0.02	0.24	2.45	0.30	1.61	0.69	0.01	0.48
Residuals	35	4.82		0.09		8.16		2.33		0.03	

770

771

772 Table 3 Warming and nitrogen-induced relative changes (%) in soil physicochemical
773 parameters during the two hydrologically contrasting years in three temperate steppes.
774 SOC = soil organic carbon, TN = total soil nitrogen, C/N = the ration of soil organic
775 carbon to nitrogen, SM = soil moisture, pH = soil pH. For significance of effects see
776 Table 2.

Variance of source	Site/year	SOC	TN	C/N	SM	pH
	meadow steppe					
warming effect	2006	2.04	18.29	-13.21	-7.57	2.85
	2007	11.18	-1.26	-0.66	-2.47	-4.70
nitrogen effect	2006	26.47	19.65	6.10	-2.40	-4.39
	2007	43.51	25.88	24.24	-13.31	-4.19
	semi-arid steppe					
warming effect	2006	-1.72	0.60	-6.20	-4.50	0.06
	2007	-2.99	-10.24	13.13	-3.24	0.17
nitrogen effect	2006	-8.09	3.38	-7.01	-8.07	-0.40
	2007	-3.09	-7.36	0.56	-4.30	-5.06
	desert steppe					
warming effect	2006	-4.23	-4.08	-0.18	-6.25	-0.47
	2007	-13.23	0.09	-13.02	-7.39	0.09
nitrogen effect	2006	1.78	1.27	3.57	2.13	-0.56
	2007	-2.91	0.62	6.86	4.19	-1.29

777

778 Table 4 Results (*F* values) of four-way ANOVA on the effects of site, warming, N
779 addition and year on main microbial groups and their ratios. Bacteria = bacterial
780 PLFA, Gram- = Gram-negative bacterial PLFA, Gram+ = Gram-positive bacterial
781 PLFA, Fungi = non-mycorrhizal fungal PLFA, AMF = arbuscular mycorrhizal fungal
782 PLFA, Gram-/Gram+ = the ratio of gram-negative to gram-positive bacteria, F/B = the
783 ratio of fungi to bacteria. The degrees of freedom (d.f.) for the numerator, mean
784 squares (MS) and mean squared error (Residuals) are shown as well. Significant code
785 ^, *, **, and *** refer to $P< 0.1$, < 0.05, < 0.01 and < 0.001.

Variance		Bacteria		Gram-		Gram+		Fungi		AMF		Gram-/Gram+		F/B	
of source	d.f.	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F	MS	
block															
site (S)	2	4.04	0.10	0.56	0.07	4.96	0.37	15.85	1.91	27.83	20.55***	0.04	1.81	0.03	
warming (W)	1	0.84	0.02	0.00	0.00	1.21	0.09	0.61	0.07	0.18	0.14	0.02	1.15	0.00	
S × W	2	1.40	0.04	0.33	0.04	0.45	0.03	0.47	0.06	0.28	0.21	0.00	0.00	0.00	
Residuals	25	39.06		7.99		13.44		8.32		1.35		0.02		0.01	
block:plot															
W	1	29.23	3.82^	3.66	1.39	10.34	3.40^	1.38	0.46	0.82	2.53	0.00	0.08	0.00	
N addition (N)	1	1.00	0.13	10.33	3.93^	4.08	1.34	1.67	0.56	0.59	1.83	0.09	4.84*	0.01	
S × N	2	1.21	0.16	0.31	0.12	1.49	0.49	2.93	0.98	0.19	0.57	0.01	0.34	0.01	
W × N	1	1.98	0.26	0.53	0.20	4.96	1.63	2.61	0.87	0.42	1.30	0.02	0.93	0.00	
S × W × N	2	0.85	0.11	0.58	0.22	1.57	0.52	2.68	0.90	0.90	2.77^	0.01	0.59	0.01	
Residuals	35	7.65		2.63		3.05		2.98		0.32		0.02		0.01	
block:year															
year (Y)	1	74.05	2.13	7.38	1.23	115.37	7.57*	8.64	1.54	0.93	1.67	0.68	24.07***	0.10	
Y × S	2	37.65	1.09	21.36	3.57*	3.78	0.25	55.62	9.88**	1.56	2.81^	0.14	4.77*	0.08	
Y × W	1	90.71	2.61	6.14	1.03	44.21	2.90	19.46	3.46^	1.19	2.14	0.01	0.23	0.01	
Y × S × W	2	12.50	0.36	2.74	0.46	2.05	0.13	6.56	1.16	0.38	0.69	0.00	0.17	0.01	
Residuals	25	34.70		5.98		15.24		5.63		0.56		0.03		0.00	
block:plot:year															
Y × W	1	17.74	1.98	0.97	0.34	10.03	3.41^	0.47	0.15	0.00	0.00	0.01	0.70	0.00	
Y × N	1	69.97	7.82**	3.90	1.38	36.29	12.32**	27.03	8.29**	1.02	3.47^	0.01	0.49	0.02	
Y × S × N	2	32.07	3.58*	2.79	0.99	13.68	4.64*	9.33	2.86^	0.44	1.49	0.01	0.45	0.01	
Y × W × N	1	3.54	0.40	7.82	2.76	0.82	0.28	0.19	0.06	0.04	0.12	0.05	3.39^	0.00	
Y × S × W × N	2	27.01	3.02^	11.52	4.07*	4.12	1.40	3.27	1.00	0.33	1.12	0.02	1.57	0.01	
Residuals	35	8.95		2.83		2.95		3.26		0.29		0.02		0.01	

786

787 Figure legends

788 Figure1. The location and long-term mean annual precipitation map of the three

789 grasslands of northern China.

790

791 Figure 2. Principal component analyses (PCA) of soil microbial community

792 composition as indicated by PLFA profiles during the two hydrologically contrasting

793 years in the three temperate steppes. Principal component (PC) 1 and PC2 accounted

794 for 40.2 % and 25 % of the total variance, respectively. The PCA ordination biplot for

795 all 24 surveyed subplots, separated for visual comparison by each steppe, showing

796 control (C, open circle), warming (W, open triangle), N addition (N, open square),

797 warming plus N addition (WN, open diamond) in 2006, and control (solid circle),

798 warming (solid triangle), N addition (solid square), warming plus N addition (solid

799 diamond) in 2007.

800

801 Figure 3. Warming and nitrogen-induced relative changes (%) in the proportion (mole

802 percentage PLFA) of main functional groups of microbial communities during the two

803 hydrologically contrasting years in three temperate steppe sites. Bacteria = bacterial

804 PLFA, Gram- = gram-negative bacterial PLFA, Gram+ = gram-positive bacterial

805 PLFA, Fungi = non-mycorrhizal fungal PLFA, AMF = arbuscular mycorrhizal fungal

806 PLFA, Gram-/Gram+ = the ratio of gram-negative to gram-positive bacteria, F/B = the

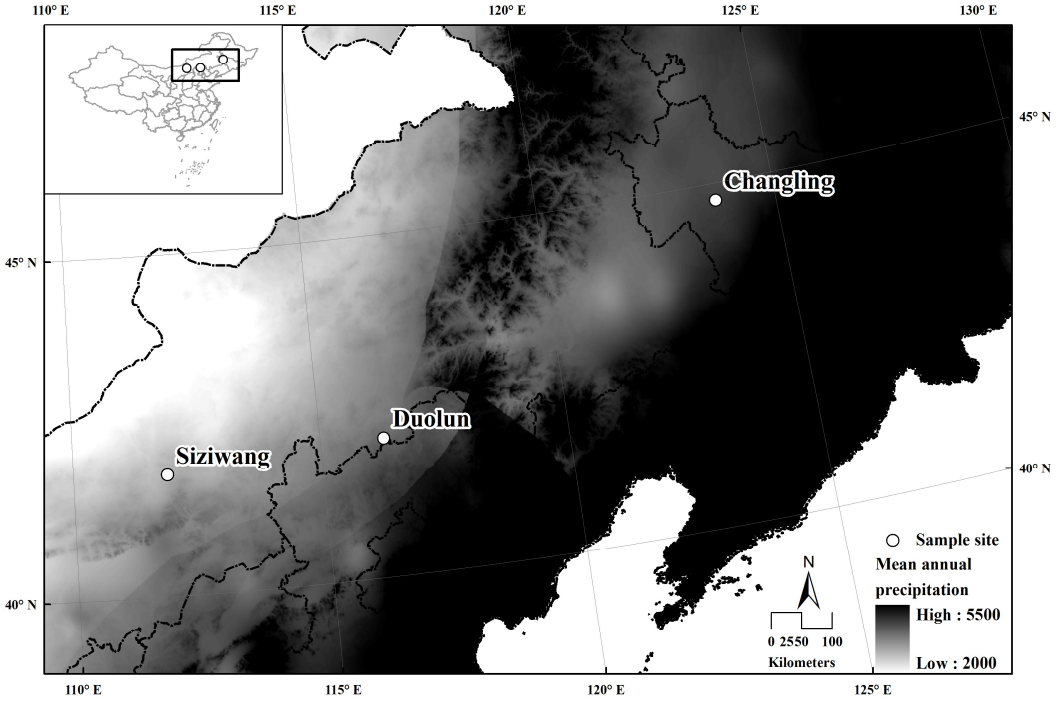
807 ratio of fungi to bacteria. For significance of effects see Table 4.

808

809 Figure 4. Principal component analyses (PCA) of soil microbial C utilization profiles

during the two hydrologically contrasting years in the meadow and semi-arid steppe sites. Principal component (PC) 1 and PC2 accounted for 75.5 % and 5.6 % of the total variance, respectively. The PCA ordination biplot for all 24 surveyed subplots, separated for visual comparison by each steppe, showing of each site exposing control (C, open circle), warming (W, open triangle), N addition (N, open square), warming plus N addition (WN, open diamond) in 2006, and control (solid circle), warming (solid triangle), N addition (solid square), warming plus N addition (solid diamond) in 2007.

819 Figure1



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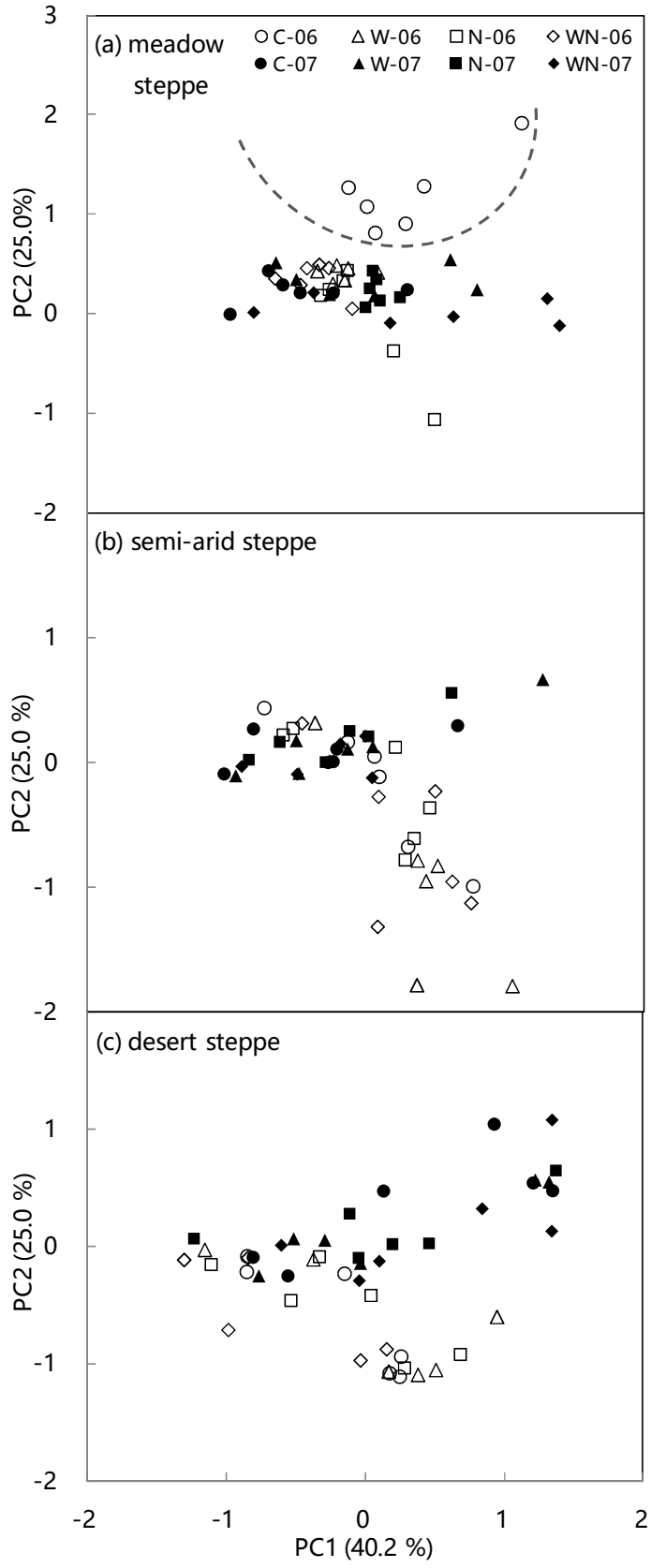
821

822 Figure2



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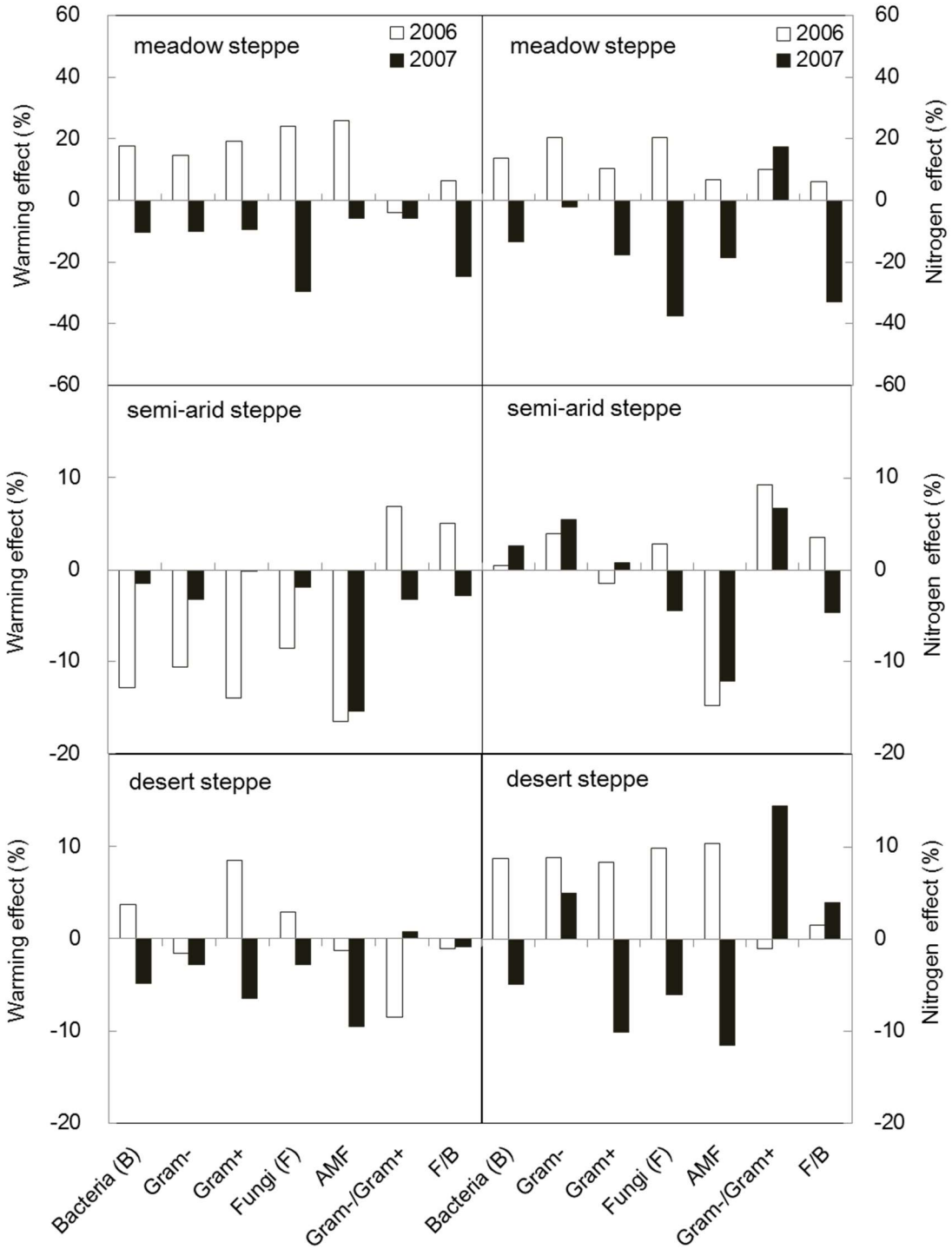
825 Figure 3



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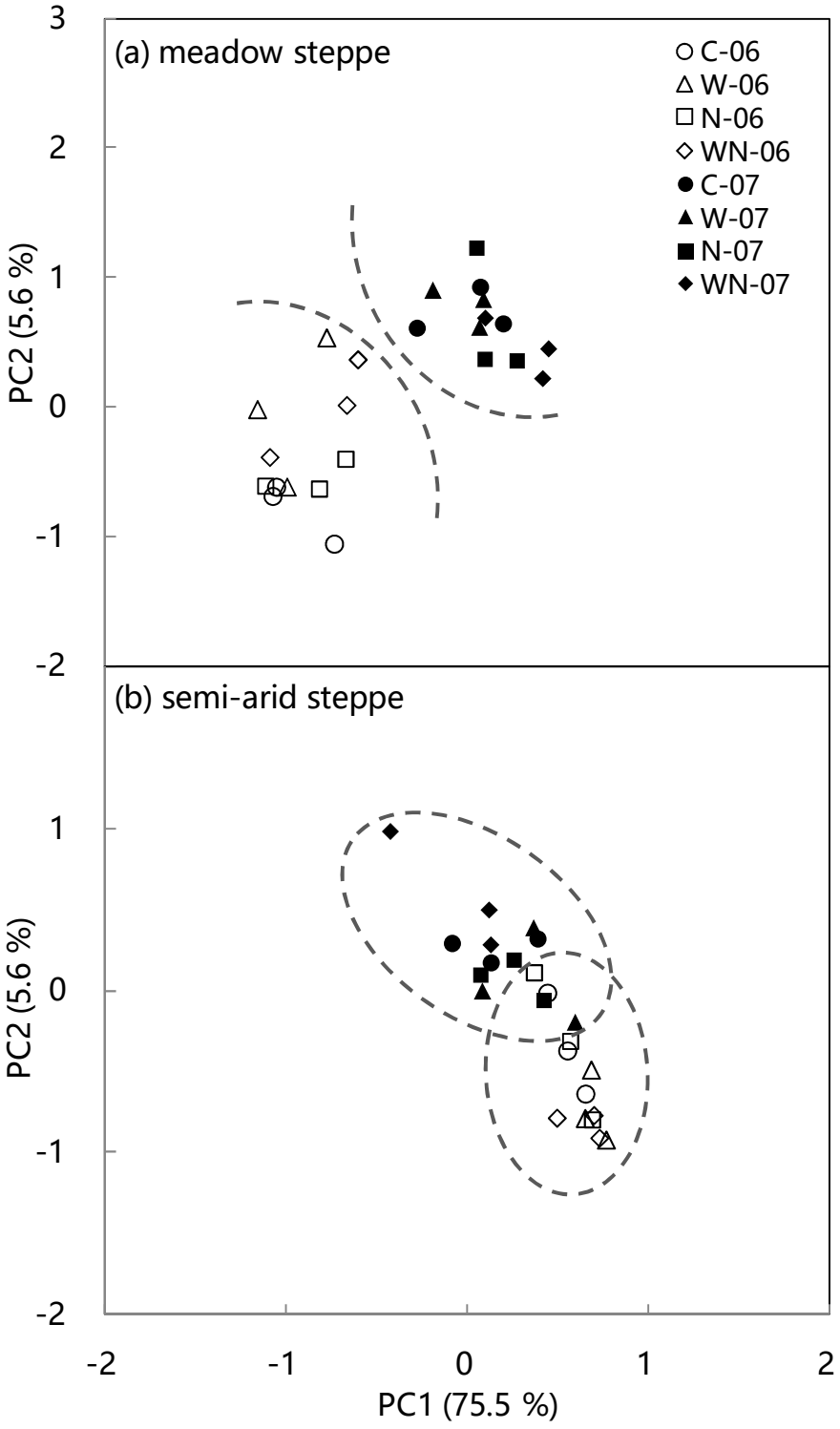
829 Figure4



830

831 Supplementary materials

832 Table S1. Differences in soil physiochemical parameters in the plots among the

833 meadow, semi-arid and desert steppe sites in 2006 and 2007 (mean ± standard error, n

834 = 24). SOC = soil organic C, TN = soil total N, C/N = the ratio of soil organic C to N,

835 SM = soil moisture, pH = soil pH. For significance of effects see Table 2.

Sites	Meadow steppe	Semi-arid steppe	Desert steppe
2006			
SOC ($g \cdot kg^{-1}$)	12.47 ± 0.69	14.29 ± 0.45	11.59 ± 0.28
TN ($g \cdot kg^{-1}$)	1.32 ± 0.08	1.40 ± 0.06	1.32 ± 0.03
C/N ratio	9.69 ± 0.33	10.49 ± 0.46	8.81 ± 0.18
SM (%)	15.49 ± 0.83	4.90 ± 0.16	6.95 ± 0.16
pH	8.93 ± 0.11	7.26 ± 0.04	7.89 ± 0.02
2007			
SOC ($g \cdot kg^{-1}$)	11.4 ± 0.70	13.63 ± 0.50	8.75 ± 0.41
TN ($g \cdot kg^{-1}$)	1.05 ± 0.09	1.32 ± 0.05	0.95 ± 0.02
C/N ratio	11.72 ± 0.80	10.64 ± 0.61	9.28 ± 0.40
SM (%)	13.02 ± 0.50	11.67 ± 0.34	4.10 ± 0.07
pH	9.27 ± 0.14	6.97 ± 0.05	8.01 ± 0.03

836

837

838 Table S2. Differences in the mole percentage PLFA (mol %) of main microbial groups
839 and their ratios in the plots among of the meadow, semi-arid and desert steppe sites in
840 2006 and 2007 (mean ± standard error, n = 24). Bacteria = bacterial PLFA, Gram- =
841 gram-negative bacterial PLFA, Gram+ = gram-positive bacterial PLFA, Fungi =
842 non-mycorrhizal fungal PLFA, AMF = arbuscular mycorrhizal fungal PLFA,
843 Gram-/Gram+ = the ratio of gram-negative to gram-positive bacteria, F/B = the ratio
844 of fungi to bacteria.For significance of effects see Table 4.

Sites	Meadow steppe	Semi-arid steppe	Desert steppe
2006			
Bacteria	21.61 ± 0.63	20.29 ± 0.87	21.44 ± 0.96
Gram-	8.36 ± 0.32	7.44 ± 0.29	8.97 ± 0.40
Gram+	12.57 ± 0.33	12.33 ± 0.63	11.97 ± 0.67
Fungi	6.00 ± 0.23	6.01 ± 0.30	7.11 ± 0.39
AMF	2.80 ± 0.16	1.42 ± 0.07	1.63 ± 0.10
Gram-/Gram+	0.67 ± 0.02	0.62 ± 0.03	0.78 ± 0.04
F/B	0.28 ± 0.01	0.30 ± 0.01	0.33 ± 0.01
2007			
Bacteria	22.35 ± 0.92	23.74 ± 0.76	21.55 ± 1.25
Gram-	7.80 ± 0.51	8.37 ± 0.32	7.24 ± 0.63
Gram+	13.81 ± 0.50	14.68 ± 0.47	13.75 ± 0.74
Fungi	5.72 ± 0.50	7.56 ± 0.53	4.37 ± 0.62
AMF	2.69 ± 0.26	1.60 ± 0.10	1.09 ± 0.15
Gram-/Gram+	0.56 ± 0.03	0.57 ± 0.02	0.52 ± 0.03
F/B	0.25 ± 0.02	0.31 ± 0.02	0.19 ± 0.02

845
846

847 Table S3 Species scores of principle component analysis on microbial C utilization

848 profiles in the meadow and semi-arid steppe (left columns) and PLFA profiles in the

849 three temperate steppes (right columns).

850

	Microbial C utilization profiles			PLFA profiles	
	PC1	PC2		PC1	PC2
Glucose-1-Phosphate	-0.70	-0.14	15:0	-0.38	0.28
DL-α-Glycerol Phosphate	-0.38	-0.06	16:1 2OH	-0.12	-0.29
D-Cellobiose	-0.98	-0.22	16:1isoG	-0.43	-0.04
α-D-Lactose	-0.58	-0.24	16:1ω5c	-0.98	0.32
Methyl β-D-Glucoside	-0.83	-0.13	16:1ω7c	-3.04	-0.63
D-Xylose	-0.79	-0.39	16:0	-0.73	3.00
i-Erythritol	-0.40	-0.09	17:1ω8c	0.10	-0.09
D-Mannitol	-0.96	0.05	17:0	-0.05	0.07
N-Acetyl-D-Glucosamine	-0.87	0.07	18:1ω5c	-0.18	0.06
D-Galactonic Acid γ-Lactone	-0.68	0.07	18:1ω9c	-2.58	-0.73
Pyruvic Acid Methyl Ester	-0.53	0.21	18:1ω9t	-0.39	-0.16
D-Glucosaminic Acid	-0.46	0.09	18:2ω6c	-0.91	-0.29
D-Galacturonic Acid	-0.75	0.47	18:0	-0.30	-0.02
γ-Hydroxybutyric Acid	-0.53	-0.08	cy19:0	-0.05	0.12
Itaconic Acid	-0.44	-0.10	19:1ω8	0.29	-1.39
α-Ketobutyric Acid	-0.29	-0.05	a15:0	-0.78	0.92
D-Malic Acid	-0.72	-0.20	a17:0	-0.41	-0.22
Tween40	-0.73	0.23	cy17:0	-0.06	0.11
Tween80	-0.59	0.20	i15:0	-1.10	0.46
α-Cyclodextrin	-0.50	-0.08	i16:0	-0.68	-0.01
Glycogen	-0.83	-0.14	i17:0	-0.43	0.03
2-Hydroxy Benzoic Acid	-0.08	0.00			
4-Hydroxy Benzoic Acid	-0.74	0.00			
L-Arginine	-0.75	0.11			
L-Asparagine	-0.78	0.34			
L-Phenylalanine	-0.27	0.03			
L-Serine	-0.71	0.07			
L-Threonine	-0.32	-0.06			
Glycyl-L-glutamic Acid	-0.30	0.06			
Phenylethylamine	-0.55	-0.12			
Putrescine	-0.46	0.09			

851

852 Figure legends

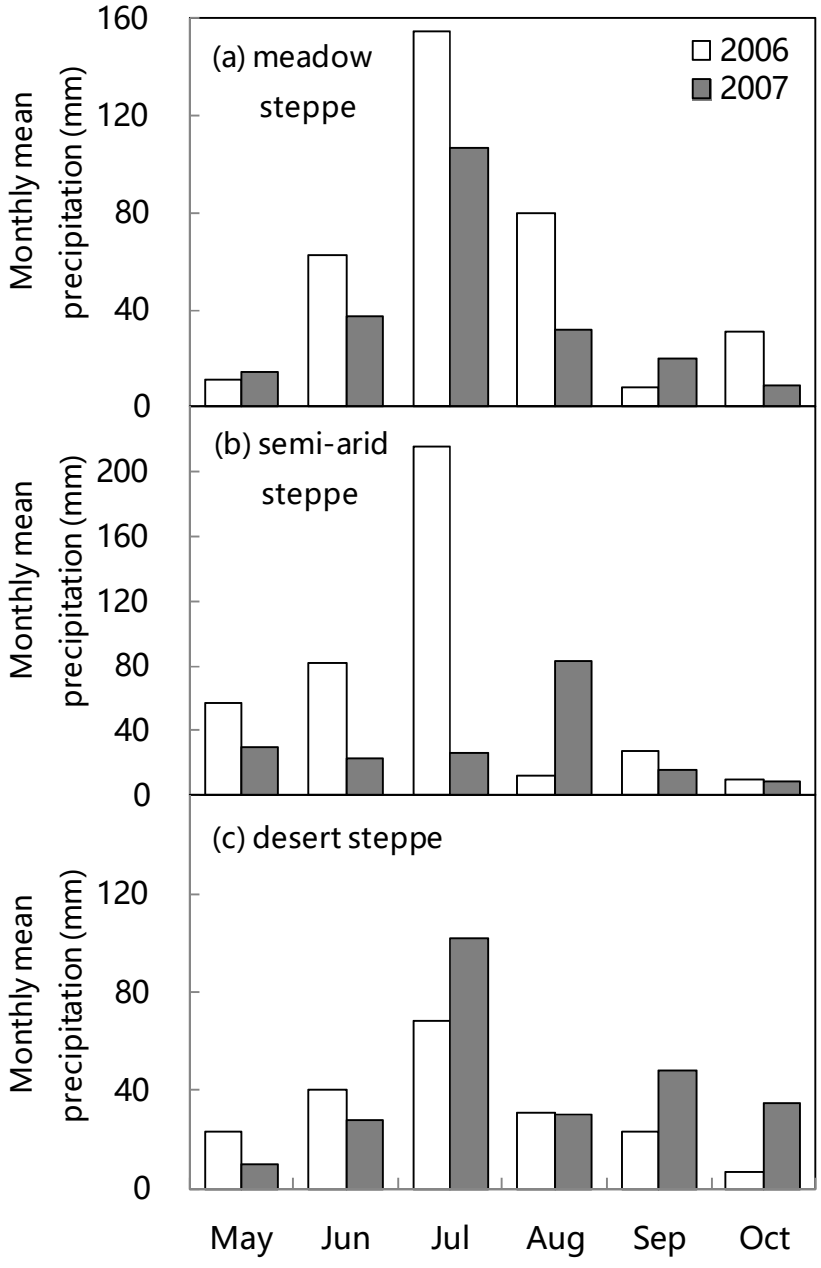
853 Figure S1. Monthly mean precipitation during the growing season of the experimental

854 years (2006 and 2007) in (a) the meadow steppe, (b) semi-arid steppe and (c) desert

855 steppe.

856

857 Figure S1



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